American Journal of Pathology, Vol. 149, No. 1, July 1996
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Expression of Most Matrix Metalloproteinase Family Members in Breast Cancer Represents a Tumor-Induced Host Response

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Matrix metalloproteinase (MMP) family members have been associated with advanced-stage cancer and contribute to tumor progression, invasion, and metastasis as determined by inhibitor studies.* In situ *hybridization was performed to analyze the expression and localization of all known MMPs in a series of human breast cancer biopsy specimens. Most MMPs were localized to tumor stroma, and all MMPs had very distinct expression patterns. Matrilysin was expressed by morphologically normal epithelial ducts within tumors and in tissue from reduction mammoplasties, and by epithelial-derived tumor cells. Many family members, including stromelysin-3, gelatinase A, MT-MMP, interstitial collagenase, and stromelysin-1 were localized to fibroblasts of tumor stroma of invasive cancers but in quite distinct, and generally widespread, patterns. Gelatinase B, collagenase-3, and metalloelastase expression were more focal; gelatinase B was primarily localized to endothelial cells, collagenase-3 to isolated tumor cells, and metalloelastase to cytokeratin-negative, macrophage-like cells. The MMP inhibitor, TIMP-1, was expressed in both stromal and tumor components in most tumors, and neither stromelysin-2 nor neutrophil collagenase were detected in any of the tumors. These results indicate that there is very tight and complex regulation in the expression of MMP family members in breast cancer that generally represents a host response to the tumor and emphasize the need to further evaluate differential functions for MMP family members in breast tumor progression. (Am J Pathol 1996, 149:273–282)

The extracellular matrix (ECM) is a complex structural and functional network, and it is generally believed that active disintegration of the basement membrane or surrounding connective tissue is required for tumor invasion and subsequent metastasis.[1] This implies that localized degradation of the ECM by enzymes released by, induced by, or activated by tumors is necessary for tumor cells to invade and migrate. Recent studies suggest that matrix degradation may also affect general cellular properties of proliferation and differentiation, thereby influencing earlier stages of tumor progression as well (see Refs. 2 and 3 for review). A clue to dissecting the roles of specific ECM-degrading enzymes in carcinogenesis may come from detailed studies of the expression patterns of these proteins during various stages of tumor progression.

The matrix metalloproteinases (MMPs) are a family of ECM-modifying enzymes associated with the malignant phenotype, and studies with natural or synthetic MMP inhibitors demonstrate that MMP activity is required for tumor progression and metastasis in several model systems (see Ref. 3 for review). The MMP family currently includes at least 11 members (see Ref. 4 for review). The collagenases are distinctive in their ability to degrade fibrillar interstitial collagens and include interstitial collagenase (IC, MMP-1), neutrophil collagenase (NC, MMP-8), and collagenase-3 (COL3, MMP-13). The gelatinases are particularly potent against denatured collagens and include gelatinase A (GELA, 72-kd gelatinase, MMP-2) and gelatinase B (GELB, 92-kd gelatinase,

Supported in part by the National Institutes of Health (RO3 CA54942 to B. Ennis and L. M. Matrisian, RO1 HD30472 to W. H. Rodgers, and R01 CA50468 to W. D. Dupont and L. M. Matrisian) and the Department of Defense (DAMD 17–94-J-4226 to L. M. Matrisian). K. J. Heppner was supported by National Institutes of Health training grant T32-CA09592.

Accepted for publication March 11, 1996.

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MMP-9). Stromelysin-1 (STR1, MMP-3), stromelysin-2 (STR2, MMP-10), and matrilysin (MAT, MMP-7) have a broad substrate specificity and degrade proteoglycans and glycoproteins such as laminin and fibronectin. Stromelysin-3 (STR3, MMP-11), metalloelastase (ME, MMP-12), and MT-MMP (MMP-14) appear to have more restricted substrate specificities.

Several MMP family members have been specifically implicated in the progression of breast cancer. For example, STR3, cloned from a subtractive screen of a breast cancer cDNA library,[5] has been shown to be expressed by fibroblasts of greater than 90% of invasive carcinomas analyzed.[5–7] Although STR3 expression has not been correlated with prognostic indicators such as tumor size, estrogen receptor level, or angiogenesis, high levels of expression of the mRNA has been associated with patient fatality due to metastatic disease.[8,9] Similarly, GELA mRNA expression and gelatinolytic activity has been associated with advanced-stage breast cancers.[6,10,11] Whereas the GELA mRNA has been demonstrated in tumor stroma, the protein has been immunolocalized to the surface of breast tumor cells themselves.[12–14] Thus, the regulation of MMP expression and activity in tumors appears complex, and the manner in which MMPs collectively contribute to breast tumor progression is not well understood.

To gain insight into potential roles of individual MMPs in mammary carcinoma progression, we have localized the mRNAs of MMP family members in a series of human breast cancer biopsies using *in situ* hybridization. The results demonstrate that MMPs are expressed in a stage- and cell-type-specific manner suggesting that individual MMPs may have specific functions and are tightly regulated during the progression of breast malignancies. These data suggest that, in general, the induction of MMPs in the stroma within tumors and in adjacent normal tissue represents a direct or indirect host response to the presence of tumor cells.

Materials and Methods

Tissue Samples

Breast biopsy specimens (n = 13) and samples from breast reduction mammoplasties (n = 12) were acquired from the surgical pathology archives of Vanderbilt University with approval from the Vanderbilt University Committee for the Protection of Human Subjects. Tissue specimens were immediately fixed in neutral-buffered formalin after removal and embedded in paraffin using an automated tissue processor. Five-micron sections were cut and mounted on Plus slides (Fisher Scientific, Pittsburgh, PA). A section of each specimen was stained with hematoxylin and eosin for histological examination.

Riboprobes

The following human MMP cDNAs were used as templates for the production of specific single-stranded RNA hybridization probes for *in situ* analysis: the 815-bp (+1 to +815) MAT cDNA,[15] the *Cla*I fragment (+1210 to +1560) of STR1,[15] the *Nde*I-*Eco*RV fragment (+1050 to +1580) of STR2,[15] the *Cla*I-*Bgl*II fragment (+1195 to +1735) of IC,[16] the 884-bp fragment (+910 to +1794) of GELA,[17] the 900-bp cDNA fragment (+852 to +1751) of GELB,[18] the 467-bp cDNA (+1127 to +1594) for STR3,[5] the 850-bp full-length cDNA for TIMP-1,[19] the *Eco*RI fragment (+1064 to +1391) of MT-MMP,[20] the 695-bp cDNA fragment (+105 to +800) for COL3,[21] the 400-bp cDNA (+1 to +405) for ME,[22] and the *Sph*I-*Kpn*I fragment (+1532 to +2032) of the NC cDNA.[23] STR3, TIMP-1, and ME cDNAs were kindly provided by Drs. P. Basset (Institut de Chimie Biologique, Strasbourg, France), G. Stricklin (Vanderbilt University), and S. Shapiro (Washington University, St. Louis, MO), respectively. NC and MT-MMP cDNAs were kindly provided by P. Cannon (Syntex Research, Palo Alto, CA), and the cDNA for COL3 was provided by J. Hambor (Pfizer, Groton, CT). Original STR1, STR2, IC, and MAT cDNAs were obtained from R. Breathnach (University of Nantes, Nantes, France), and GELA and GELB cDNAs were provided by G. Goldberg (Washington University, St. Louis, MO). All cDNAs were subcloned into pGEM vectors (Promega, Madison, WI) except GELA, GELB, and STR3, which were subcloned into Bluescript vectors (Stratagene, La Jolla, CA). Linearized templates and [^{3}H]UTP (Dupont New England Nuclear, Boston, MA) were used to generate sense and antisense hybridization probes to specific activities of approximately 10^8 dpm/μg as described.[24] Reduction mammoplasty tissues were probed with the MAT riboprobes generated using [^{35}S]UTP (Dupont New England Nuclear) as described.[15]

In Situ *Hybridization*

In situ hybridization on all breast biopsy specimens was performed as previously described.[24] Tissues were hybridized using a specific activity of 3×10^5 to 3.5×10^5 cpm/slide, and all slides were exposed for 6 weeks at 4°C and counterstained with hematoxylin.

Table 1. *Matrix Metalloproteinase Expression in Human Breast Cancers*

Metalloproteinase	Number of positive/ total cases	Number of positive/total areas analyzed			Comments
		Normal	CIS	Invasive cancer	
Epithelial					
MAT	11/13	6/9	4/4	4/11	Focal expression Also in fibroblasts in CIS
Fibroblastic					
STR1	3/13	0/9	0/4	3/11	Focal expression
STR3	11/13	0/9	2/4	9/11	Localization to fibroblasts adjacent to tumor cell nests
GELA	13/13	1/9	2/4	11/11	Diffuse expression
MT-MMP	12/13	0/9	1/4	11/11	Diffuse expression
IC	4/13	0/9	1/4	3/11	Focal expression
Other					
GELB	12/13	0/9	2/4	10/11	Localization to endothelial and inflammatory cells
ME	5/13	0/9	1/4	4/11	Localization to isolated macrophages and necrotic areas
COL3	4/13	0/9	1/4	3/11	Localization to isolated tumor cells
TIMP-1	11/13	0/9	4/4	9/11	Localization to stromal and tumor components

Human endometrium samples were used to confirm the specificity of most probes and as positive controls as most MMPs display characteristic expression patterns as described previously.[25] *In situ* hybridization using [^{35}S]UTP on breast reduction tissue was performed as previously described.[15] Slides were hybridized at a specific activity of 1.2×10^6 cpm/slide, and they were exposed at 4°C for 4 weeks. A breast biopsy specimen determined to be positive for MAT mRNA in earlier experiments was used as a positive control. In all experiments, hybridization intensity and distribution were analyzed using dark-field microscopy and were scored relative to background hybridization on sense control slides. Hybridization signals were independently scored by at least two observers. For several biopsy samples, coverslips were removed from *in situ* slides, and the tissues were stained with an anti-human cytokeratin (CAM5.2) monoclonal antibody (Becton Dickinson, San Jose, CA) by routine pathological techniques to identify epithelial-derived tumor cells.

Results

In situ hybridization was performed on 13 cases of human breast cancer using riboprobes specific for all of the known MMP family members and an endogenous MMP inhibitor, TIMP-1. The MAT, STR1, STR2, STR3, GELA, GELB, and IC riboprobes have been shown to be specific for individual MMPs in human endometrium[25] and colorectal tumor[24] specimens. Similarly, in this study, hybridization patterns of the COL3, ME, MT-MMP, and NC probes indicated that they did not cross-react with each other or with other MMP family members. In addition, all specimens demonstrated specific hybridization with at least one probe. Areas of each biopsy, including morphologically normal areas associated with the tumor, carcinoma *in situ* (CIS), and invasive cancer, were evaluated independently for hybridization with specific antisense probes as compared with sense probe controls. The *in situ* hybridization results are summarized in Table 1.

MAT was the only MMP with widespread localization to epithelial components of the tumors and was detected in the majority (11/13) of the specimens examined (Table 1). In several cases (6/9), MAT was localized to the epithelium of morphologically normal ducts and lobules adjacent to the primary tumor (see Figure 1A for a representative sample). In areas of CIS, we observed two distinct patterns of MAT expression; MAT mRNA was detected in focal tumor nests in all of the CIS samples examined (Figure 1C for example) whereas in 2 of the 4 samples, the MAT probe hybridized in a focal pattern to fibroblasts directly adjacent to tumor cells (Figure 1B, for example). MAT mRNA was expressed by invading tumor cells in a sporadic pattern in a few (4/11) of the invasive breast cancers analyzed (Figure 1D, for example).

Most MMP family members were expressed in many of the invasive cancers with widespread localization to fibroblastic cells throughout the tumor stroma. STR3 and GELA were expressed in nearly all of the invasive cancers analyzed (Table 1), which is consistent with previous reports.[5–7] Although the mRNA for both MMPs was localized to fibroblasts within the stroma, their patterns of expression were

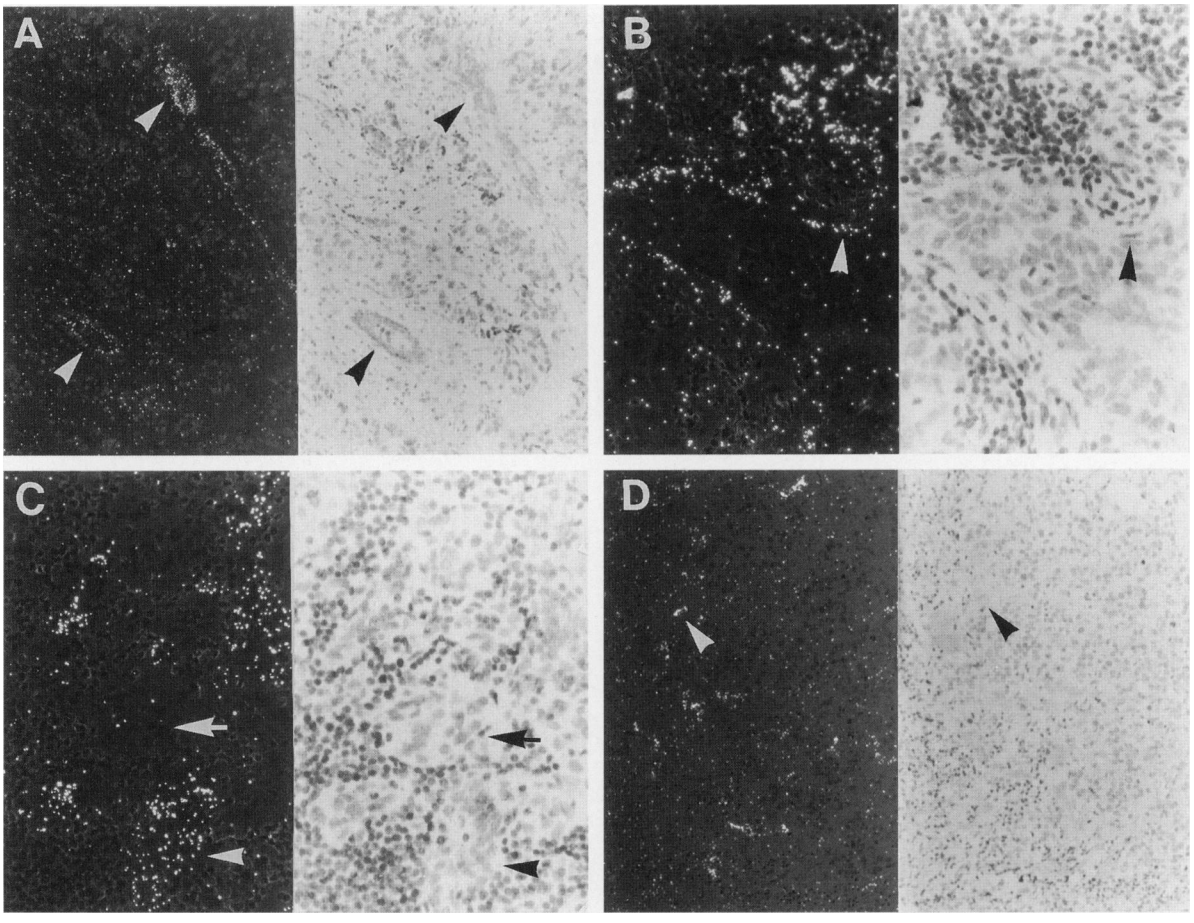

Figure 1. *MAT localization in breast cancer specimens.* A: *MAT is expressed in the epithelium of a morphologically normal duct entrapped by tumor. Magnification, ×200.* B: *An area of carcinoma* in situ *(CIS) expresses MAT in those fibroblasts immediately adjacent to tumor cell nests. Magnification, ×500.* C: *An area of the same CIS specimen as in* A *demonstrates MAT localization to nests of tumor cells* (arrowhead). *Occasional nearby tumor cells are negative for MAT expression* (arrow). *Magnification, ×500.* D: *An invasive cancer expresses MAT focally in isolated tumor cells throughout the specimen. Magnification, ×320. Dark-field illumination is shown on the left with its corresponding bright-field image on the right. All* in situ *hybridizations were counterstained with hematoxylin.*

distinct. STR3 mRNA was detected in those fibroblasts immediately adjacent to the malignant epithelium (Figure 2A depicts a representative sample). GELA, however, was diffusely present in stromal fibroblasts throughout and surrounding the tumor (Figure 2B, for example). Similarly, MT-MMP mRNA was identified in all cases of invasive cancer in a diffuse pattern in stromal fibroblasts of the cancers (Figure 2C, for example), with significant but imperfect co-localization with GELA mRNA. IC and STR1 mRNAs were expressed by tumor-associated fibroblasts in a very focal pattern (Figure 2D, for example). In addition to being expressed in limited areas of these tumors, a very limited number of tumors expressed IC (3/11) or STR1 (3/11) mRNAs (Table 1). One sample of infiltrating ductal carcinoma expressed both of these mRNAs, as well as the mRNA for GELA, MT-MMP, STR3, and MAT.

Other MMPs were expressed sporadically by isolated cells in the invasive cancers analyzed. GELB was expressed in the majority of the tumors (12/13 cases) but was localized primarily to either stromal endothelial cells (Figure 3A, for example) or isolated infiltrating lymphocytic or macrophage-like inflammatory cells. COL3 mRNA was detected in 3/11 cases with a focal but intense hybridization signal in isolated tumor cells (Figure 3B, for example). This localization was verified using cytokeratin immunohistochemistry on the same slide (data not shown). A similar focal, intense hybridization pattern was observed with the ME cDNA probe in 4/11 tumors (Figure 3C, for example). ME mRNA was also expressed in areas of tumor necrosis (data not shown), and the cells demonstrating ME hybridization did not stain positive for cytokeratin (data not shown). Based on the previously re-

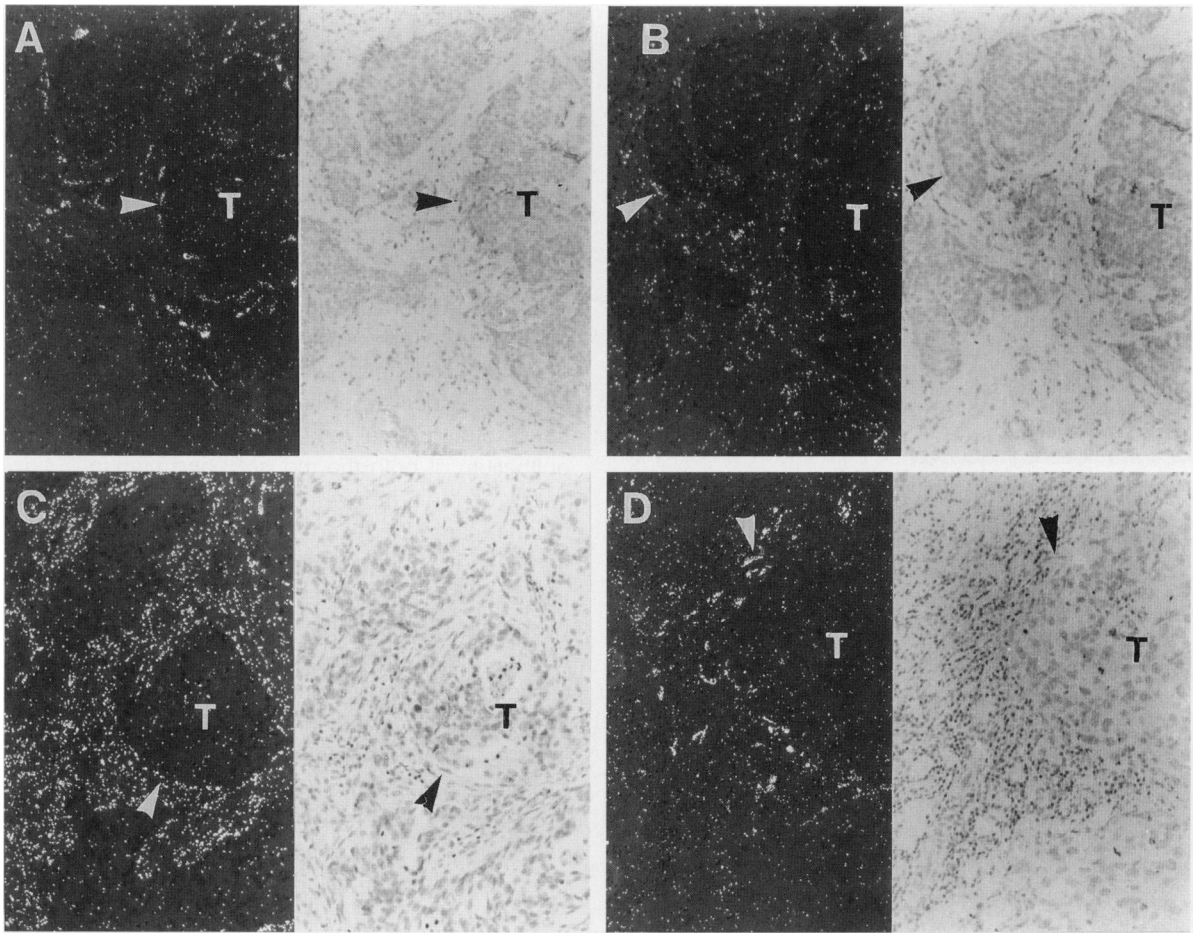

Figure 2. *Localization of fibroblastic MMPs in invasive breast cancers.* A: *STR3 is localized to fibroblasts adjacent to tumor cells (T) in an invasive cancer. Magnification, ×200.* B: *GELA localization in an adjacent section of the cancer shown in* A *in fibroblasts throughout the tumor-associated stroma. Magnification, ×200.* C: *MT-MMP is expressed diffusely by fibroblasts throughout the stroma of an invasive cancer. Magnification, ×200.* D: *IC is localized to isolated fibroblasts and clusters of fibroblasts throughout the stroma. Magnification, ×200. Corresponding dark- and bright-field images are shown, and counterstain is hematoxylin.*

ported restricted expression of ME in macrophages,[22,26] we favor the interpretation that ME transcripts are primarily localized to isolated macrophages.

The expression of stromal MMPs differed from the expression of MAT in that they were detected rarely in morphologically normal areas of tissue adjacent to the tumors and occasionally in areas of CIS (Table 1). Neither STR2 nor NC were expressed at detectable levels in any of the tumors analyzed. The mRNA of an endogenous inhibitor of MMPs, TIMP-1, was detected in many of the invasive cancers, with localization predominantly but not exclusively to tumor stroma (Table 1). Experiments performed with TIMP-2 were inconclusive due to high levels of background hybridization using sense riboprobes. Two new MT-MMPs[27,28] were described while this manuscript was in preparation and have not been included in this study.

As MAT was the only MMP frequently expressed in morphologically normal breast tissue, we examined 12 cases of tissue obtained at the time of breast reduction mammoplasty. All 12 cases demonstrated MAT expression in some, but not all, glandular structures (data not shown).

There was no obvious correlation between the expression patterns of any of the MMP family members in either areas of adjacent normal and CIS or areas of adjacent normal and invasive cancer. For example, in two cases that contained normal and CIS without evidence of invasive cancer, MAT was expressed in the neoplastic areas in both instances but was associated with morphologically normal structures in only one of two specimens (Table 2). In addition, there was no definitive correlation between the expression of any MMP family member with another. GELA and MT-MMP, for example, were expressed in all cancers examined (Table 1) but were

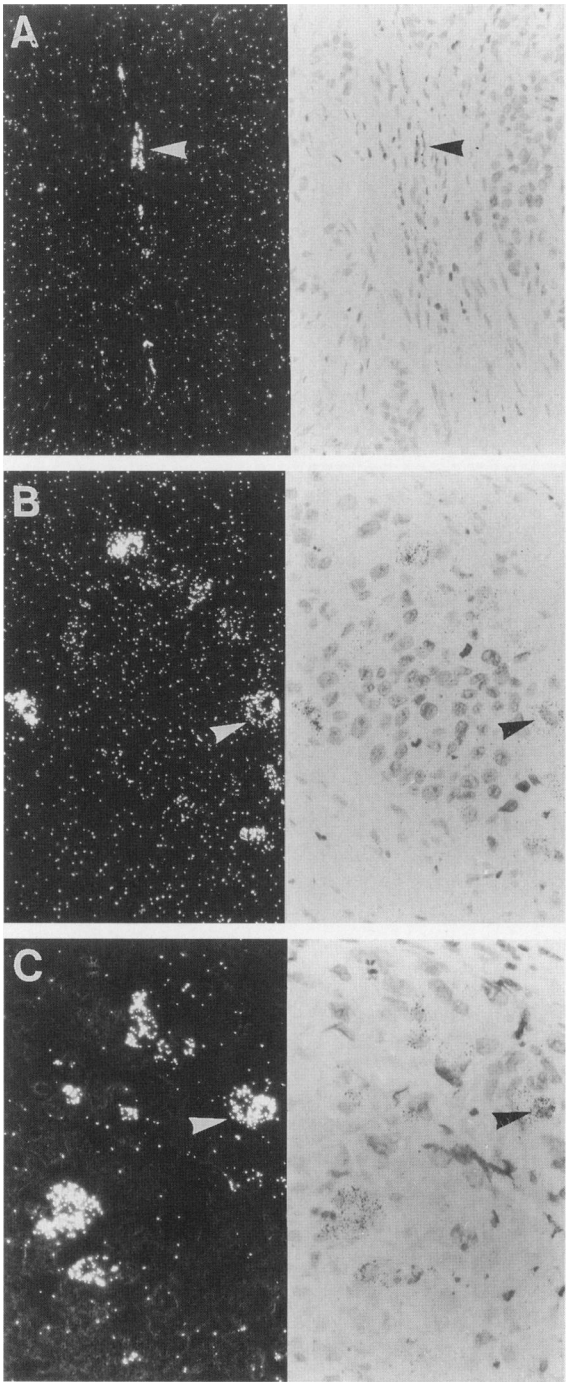

Figure 3. *Non-fibroblastic localization of GELB, COL3, and ME in invasive breast cancers.* **A:** *GELB is expressed in endothelial cells of a blood vessel running through an invasive cancer. Magnification, ×320. COL3* (**B**) *is expressed by isolated tumor cells and ME by isolated macrophages* (**C**) *in the same specimen. Magnification, ×320 and ×500, respectively. Corresponding dark- and bright-field images are shown, and counterstain is hematoxylin.*

not necessarily co-expressed in cases of CIS (see case 1, Table 2). The small number of cases and the complexity of breast tumor progression precludes an evaluation of the potential functional significance of these expression patterns relative to the biology of the disease.

Discussion

Expression patterns of known MMP mRNAs in human breast cancer specimens show distinct cell and tissue localization and suggest specific regulation and unique functions for these enzymes in breast tumor progression. Although MAT is the only MMP detected in the epithelium of morphologically normal tissues, its localization is not entirely surprising because of its normal expression in various glandular epithelium of both the mouse[29] and human.[30] Low levels of MAT mRNA have been detected in the murine mammary gland during lactation and involution,[29] and in our analysis of MAT in breast reduction tissue, the mRNA was localized heterogeneously to hyperplastic breast epithelium. In addition, the protein has been immunolocalized to fibrocystic breast tissue and benign fibroadenomas.[30] This suggests that, although it is unclear whether MAT is expressed in normal, resting breast epithelium, its expression is perhaps indicative of structural changes, either physiological or in response to tumor, that have occurred within the mammary environment. It is possible that MAT expression is regulated by tumor or stromally derived factors present in the remodeling breast environment. The presence of MAT in a few morphologically normal ducts may indicate that structural changes have occurred within the epithelium, which is a notion supported by the presence of MAT in all of the CIS samples analyzed.

MAT is the only MMP detected in the epithelium of both benign and malignant tumors. MAT mRNA was detected in many but not all tumor cell nests in areas of CIS and in some stromal fibroblasts immediately adjacent to tumor cells in the same specimens. Although MAT has been described previously in benign lesions of the colon[24,31] and breast,[5,6] fibroblastic expression of MAT mRNA in such tumors has not been reported. It is possible that this localization represents patient and tumor heterogeneity in the expression or localization of potential MAT regulators or in mammary fibroblast responsiveness to such regulators. Evidence supporting the localization of MAT to fibroblasts includes the demonstration that MAT expression can be induced in human gingival fibroblasts under certain conditions.[32] Consistent with previous analyses of MAT in cancers of the colon,[24,31] prostate,[33] lung,[34] and breast,[5,6] MAT expression was detected in the tumor cells of some but not all invasive cancers. Tumor heterogeneity,

Table 2. *Expression of Selected MMPs in Breast Cancer Cases*

	Case 1		Case 2		Case 3		Case 4		Case 5		Case 6		
	N	CIS	N	CIS	N	CA	N	CA	N	CA	N	CIS	CA
MAT	−	+	+	+	+	−	−	+	+	−	+	+	−
GELA	−	+	−	+	−	+	−	+	+	+	−	−	+
STR3	−	+	−	+	−	+	−	+	−	+	−	−	+
MT-MMP	−	−	−	+	−	+	−	+	−	+	−	−	+

N, normal; CIS, carcinoma *in situ*; CA, invasive cancer.

small sample size, or reduced sensitivity of the assay using ^{3}H-labeled riboprobes may be responsible for a lower percentage of positive invasive breast cancers in this study than previously demonstrated.[5,6]

It is somewhat ironic that MMP expression in the malignant epithelium of breast cancers is apparently limited to sporadic expression of MAT and COL3 as MMPs are known to be transcriptionally regulated by oncogenes, and it was widely assumed that MMP expression in tumor tissue was the direct result of transforming events.[35] The expression of MAT throughout the epithelium of early-stage tumors suggests the possibility that MAT may play a role in early stages of mammary carcinoma formation, perhaps similar to the effects on tumor establishment and growth observed in colorectal cancer.[36] Other features also distinguish MAT from other MMP family members, including the lack of the carboxyl-terminal hemopexin-like domain, its expression in normal epithelium, and its association with organogenesis.[37] It is likely that MAT has a role in normal tissues and breast cancer tumorigenesis distinct from that of other MMP family members. COL3 was detected in isolated tumor cells of invasive cancers, which is consistent with its isolation from a breast-tumor-derived cDNA library and immunolocalization to breast tumor cells.[21] Although a role for COL3 in breast tumor progression remains only speculation at this time, its expression suggests a more localized function in the microenvironment of individual tumor cells in late-stage tumors.

From the data presented in this study, the expression of the majority of MMP family members is most consistent with regulation via a stromally derived host response to the presence of neoplastic cells. Stromal fibroblasts were shown to produce STR3, GELA, and MT-MMP in nearly all invasive cancers but not in the same places throughout the tissues and only occasionally in areas of CIS. Whereas STR3 was consistently detected in those fibroblasts directly adjacent to nests of malignant cells as has been shown previously,[6] GELA and MT-MMP were more diffusely localized throughout the stroma within and surrounding the tumor. IC and STR1, also expressed by tumor-associated fibroblasts, demonstrated a more focal pattern of localization in only a few tumors. These results suggest that regulation of MMP expression in fibroblasts is clearly distinct among family members. STR3, GELA, and MT-MMP transcription may be responsive to different signaling pathways governed by various diffusable factors. Alternatively, STR3 expression in fibroblasts, for example, may be regulated by the same factor as GELA expression, but the response of the cell is dependent on the concentration of the factor presumably secreted by the tumor cells. In this scenario, higher levels of STR3 would be generated in those fibroblasts at the tumor-stroma interface than the more diffusely expressed GELA. Such subtle differences may be important as stromal MMPs are likely to play a significant role in the invasion step of tumor progression (see Ref. 3 for review).

Fibroblasts are not the only stromal cells that synthesize MMP mRNAs in breast malignancies. Interestingly, endothelial cells of blood vessels infiltrating the invasive cancers expressed GELB in several specimens. GELB was also detected in inflammatory cells, which has been reported in previous studies.[6] GELB appears to be uniquely expressed and regulated in endothelial cells and can be postulated to play a role in angiogenesis. The very focal expression of ME in isolated macrophages suggests that these cells may produce the enzyme locally as a wound-like response to the presence of invasive tumor cells. Most stromal MMPs were occasionally expressed in areas of CIS. However, because of the small number of samples, which represent various types of CIS, no conclusions can be drawn concerning the significance of MMP expression in pre-malignant lesions and potential functional consequences on their expression.

Although there is a predominance of stromal MMP expression in the analyzed tumors, all of the MMP family members are expressed in unique patterns. This suggests that, although many of the MMP promoter elements are well conserved among family members, specificity must exist to differentially regulate their expression. In addition to regulation by

oncoproteins, MMP expression has been shown to be regulated by growth factors and cytokines, tumor promoters, hormones, and ECM components. Studies of ECM remodeling in normal systems, such as the human endometrium, have provided insight into potential modes of MMP regulation and function in cancer. For example, patterns of MMP expression in the endometrium implicated roles for steroid hormones in MMP regulation and indicated potential differential functions of family members in normal ECM remodeling.[25] Of particular interest and relevance to carcinogenesis, the expression of MMPs usually considered to be associated with tumor progression in the colon, prostate, and breast, ie, MAT, STR3, and the GELA, were particularly abundant and dynamically expressed in normal endometrium. It is important to consider possible MMP regulation by steroid hormones in mammary tumorigenesis, as the breast is a similarly hormone-responsive tissue. The precise signaling pathways mediating MMP regulation by hormones is not well understood but have been shown to involve direct interaction with AP-1 transcription factors in some cases[38–40] and indirect effects through growth-factor-mediated pathways in other cases.[41] Although AP-1 transcription factors are presently the most likely candidates to provide downstream regulation of MMP transcription in many cell types, it is clear that much work is necessary to dissect the complex pathways regulating MMP expression in both normal and neoplastic situations.

In addition to the obvious complex regulation of MMP transcription, localization of protease activity adds even more complexity to the story of MMPs in tumor progression. For example, the serine protease urokinase plasminogen activator (uPA) specifically binds to a cell surface receptor present on tumor cells (see Ref. 42 for review). Likewise, GELA binds to and can be activated by the recently described membrane-bound MT-MMP.[20] Although MT-MMP mRNA has been localized to stromal fibroblasts in several tumor types,[43] the protein has been immunolocalized to the surface of tumor cells.[20] This mechanism for localized activation of proenzymes is particularly intriguing for GELA, which is known to be present in abundance throughout normal and neoplastic ECM remodeling. In addition, the apparent relative lack of transcriptional regulatory activity for GELA in tissues suggests that membrane-associated activation of the proenzyme may be a key event in its regulation. Similarly, activation of uPA may initiate a proteolytic cascade via production of plasmin, a broad-spectrum protease with activity against many ECM components and pro-MMPs.[1] Although the activity of serine proteases, including uPA, may contribute to MMP activation, the presence of tissue inhibitors of metalloproteinases (TIMPs) can counterbalance such activation and provide an additional level of MMP regulation. As shown in this study, TIMP-1 is often overexpressed in a high percentage of tumors and in the same cell types that produce MMPs. Recently, TIMP-3 has been cloned from a breast-cancer-derived cDNA library and was detected at high levels in primary breast tumors.[44] TIMP-2 has also been reported to be overexpressed in a high percentage of breast carcinomas, and its expression in such cases was shown to actually correlate with tumor recurrence.[45] It is possible that TIMP overexpression is the host response to tumor invasion in an attempt to control MMP activity and retain ECM integrity.

It is useful to approach ECM remodeling in pathological processes from a tissue homeostasis perspective rather than the study of an individual enzyme. It is clear that MMP family members are regulated distinctly and perhaps have very different functions. At the present time, synthetic inhibitors of general MMP activity have been shown to be effective therapeutic agents for ovarian, colon, and endothelial cell tumors in mouse model systems.[46–48] The timing of the delivery of these inhibitors may be critical, and it is even possible that in certain situations general inhibitors may ablate normal host defenses and exacerbate the progression of the tumor. With the development of second generation inhibitors with specificity for individual MMP family members (see Ref. 49, for example), one may be able to target specific events in breast tumor progression associated with specific MMPs. Additional studies into the expression and role of specific MMPs in the various stages of tumor progression will help guide the therapeutic application of these compounds.

Acknowledgments

The authors thank Dr. Bruce Ennis for his involvement in initiating these studies, Chris Svitek, Kim Newsom, and Sandy Olsen for excellent technical assistance, and Jane Wright for helpful assistance with photography.

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